

BQS

12

# EUROPEAN PATENT APPLICATION

21 Application number: 83303193.3

51 Int. Cl.<sup>3</sup>: G 01 N 27/30, G 01 N 27/28

22 Date of filing: 02.06.83

30 Priority: 02.06.82 JP 94575/82

71 Applicant: FUJI PHOTO FILM CO., LTD., 210 Nakanuma Minamishigara-shi, Kanagawa-ken (JP)

43 Date of publication of application: 07.12.83  
Bulletin 83/49

72 Inventor: Kitajima, Masao, 11-46, Senzui 3-chome, Asaka-shi Saitama (JP)  
 Inventor: Seshimoto, Osamu, 11-46, Senzui 3-chome, Asaka-shi Saitama (JP)  
 Inventor: Kubotera, Kikuo, 11-46, Senzui 3-chome, Asaka-shi Saitama (JP)  
 Inventor: Yamaguchi, Akira, 11-46, Senzui 3-chome, Asaka-shi Saitama (JP)

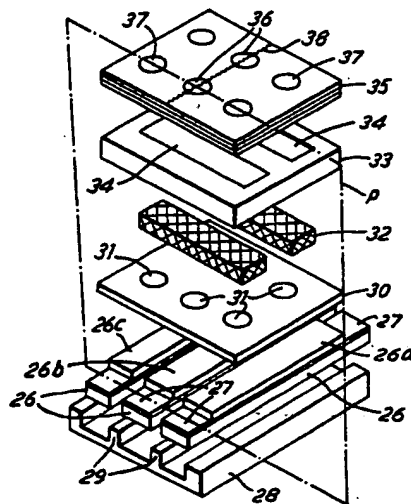
24 Designated Contracting States: DE FR GB

74 Representative: Diamond, Bryan Clive et al, Gee & Co. Chancery House Chancery Lane, London WC2A 1QU (GB)

54 Device for determining ionic activity.

57 A device for determining ionic activity is composed of plural pairs of ion-selective solid electrodes (26) each pair having two electrodes (one electrode on each of two sides) and each pair having a different type of ion-selective layer (26a, 26b, 26c); two liquid distribution porous members (32), each member being disposed over all the electrodes (26) or each said side of the plural pairs; and a porous capillary bridge (38) disposed on or between the liquid distribution porous members. The bridge (38) can be in liquid contact with all the electrodes (26) through the liquid distribution porous members (32) and achieve electrical conduction between two electrodes in each pair after spotting a test solution and a reference solution. The device preferably further comprises a support from (28) and a cover (35) having two apertures (36) for liquid spotting.

The device can simultaneously determine ionic activities of a plurality of different ions in a test solution, such as whole blood, plasma, serum or urine, by spotting a test solution and a reference solution each in one operation and measuring the electrical potential difference of each pair.



EP 0 095 946 A1

## DEVICE FOR DETERMINING IONIC ACTIVITY

This invention relates to a device for the determination of concentrations or activities of specific ions contained in a liquid or a solution. More particularly, the invention relates to a device for  
5 determining ionic activities comprising ion-selective solid electrodes (hereinafter, these are sometimes referred to as solid electrodes) for potentiometrically measuring ion concentrations or ionic activities (hereinafter,  
10 they are referred to as ionic activities) of, in particular, aqueous liquids or solutions in body fluids of a living body, such as whole blood, blood plasma, serum, lymph, spinal fluid or urine, said device being capable of determining ionic activities of different  
15 ions contained in a same single test liquid or test solution (liquid analyte) simultaneously or successively by one operation.

Conventional techniques use a wide variety of devices for determining ionic activities in solutions  
20 such as dry type ion-selective electrodes. The methods of using these electrodes are determined from the viewpoints of easiness in maintenance, storage and handling during measurement as well as of low cost. For example, rod-

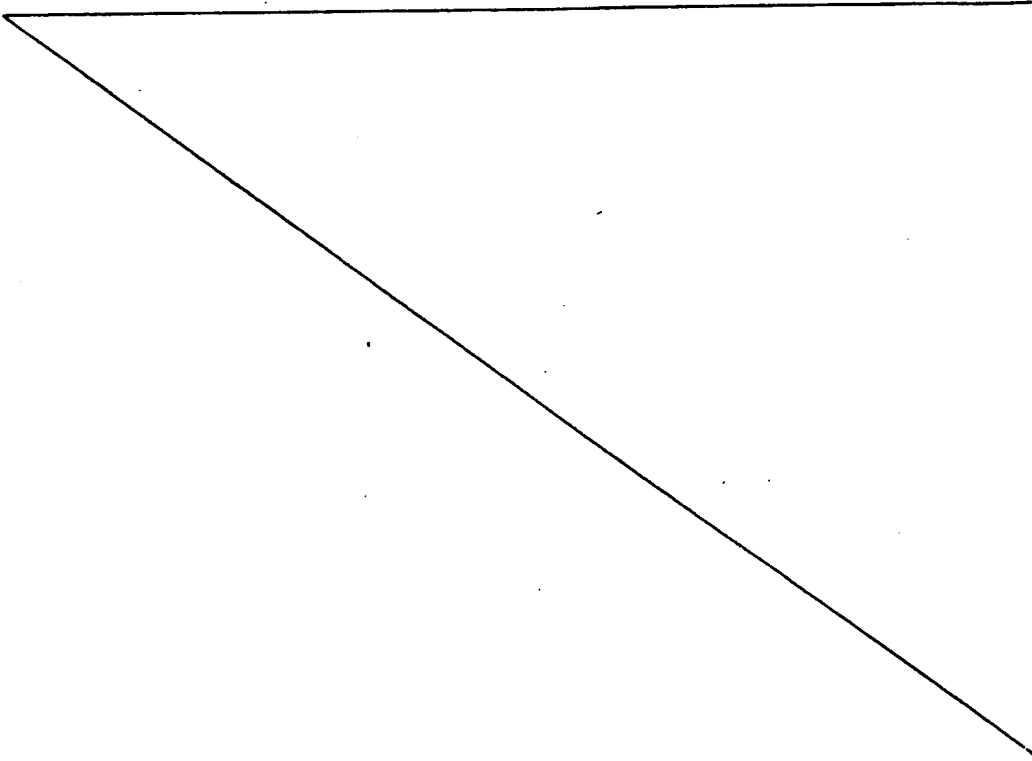
form or probe-form ion-selective solid electrodes are disclosed in U.S. Patent 4,115,209, Japanese Utility Model Publication No. 14472/65, U.S. Patent 3,718,569,

Also, ion-selective solid electrodes each composed  
5 of a support having formed thereon a laminate structure of four-functional layers, a laminate structure of three-functional layers, or a laminate structure of two-functional layers as film-shaped structure are disclosed in, for example, Research Disclosure, No. 16113 (September  
10 1977), U.S. Patents 4,053,381, 4,115,209, 4,214,968, and Japanese Patent Applications (OPI) Nos. 17851/82 and 17852/82 (the term "OPI" as used herein refers to a published unexamined Japanese patent application ). Such an ion-selective electrode is generally composed of  
15 a structure comprising an electrically insulating support having laminated thereon, in succession, a metal layer, a layer of a water-insoluble salt of the metal same as the metal of the aforesaid metal layer, a dried reference electrolyte layer composed of a hydrophilic  
20 binder matrix having dissolved therein a water-soluble salt having a common anion to the water-insoluble salt layer (a dried reference electrolyte layer is omitted in the foregoing three-functional laminate layer type ion-selective electrode), and an ion selective layer.  
25 Furthermore, U.S. Patent 4,115,209 discloses ion-

selective electrodes of a two-functional layer laminate structure wherein a conductive material is coated with an ion-selective layer containing an ion-exchange material.

5                Devices for determining ionic activity using such ion-selective electrodes include a device composed of a pair of juxtaposed ion-selective electrodes and a capillary bridge (hereinafter, is simply referred to as a bridge) for attaining electrical conduction between the  
10 electrodes by being formed on these electrodes to electrically connect them. A determination method using the device is performed by spotting a test solution and a reference solution onto definite positions, respectively, of the electrodes to cause electrical conduction  
15 between both solutions through the bridge and measuring the potential difference occurring between the electrodes by means of a potential measuring device. That is, the solutions spotted on the surfaces of the electrodes wet the surfaces thereof and at the same time diffuse into  
20 the bridge disposed on the electrodes by a capillary phenomenon. The solutions are brought into contact with each other at a thin contact interface, whereby electric conduction is attained between the electrodes to make it possible to measure the potential between the  
25 electrodes. In this case, each ion-selective electrode

has an ion-selective layer selectively responsive to a predetermined specific ion as the outermost layer. The ion-selective layer is a layer which is generally composed of an ion carrier, a solvent for the ion carrier, and a polymer binder. The ion-selective layer can be selectively responsive to a specific ion by selecting the ion carrier and the solvent for the ion carrier. This means that in the case of determining the ionic activity of each of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , etc., in, for example, serum solid electrodes, each having a different specific ion-selective layer (the layer may be an ion-permeable protective layer described below in case of determining the ionic activity of  $\text{Cl}^-$ ) must be separately prepared.



Th device f the present invention can determin  
ionic activities of plural items in one operation  
since in this invention a test liquid or a test solution  
(hereinafter referred to as test solution) and a  
5 reference solution can be supplied to the surfaces of plural  
pairs of electrodes by spotting the test solution and  
the reference solution each only once.

An object of this invention is to provide a  
device for determining ionic activity wherein by only  
10 spotting each of a test solution and a reference solution  
onto at least each one of independent liquid distribution  
porous members each disposed on plural pairs of solid  
electrodes, each electrode pair having one electrode on  
each of two sides, so that each liquid distribution  
15 porous member covers at least partially all the

---

electrodes at each said side of the electrode pairs, the solutions are supplied to the surfaces of the plural pairs of the solid electrodes each having a different ion-selective layer, whereby ionic activities of plural  
5 items can be simultaneously or successively determined by one operation.

Another object of this invention is to provide a device for determining ionic activity capable of certainly performing the distribution and contact of  
10 solutions even if a test solution having a high viscosity, such as whole blood, etc., is used.

According to this invention, there is provided a device for determining ionic activity composed of plural pairs of ion-selective solid electrodes each pair  
15 having an ion-selective layer selectively responsive to a predetermined specific ion as the outermost layer of the solid electrode and a porous capillary bridge for achieving, after supplying a test solution and a reference solution to said pairs of solid electrodes, the  
20 electric conduction of both solutions by the permeation of the solutions through the porous capillary bridge, wherein said device comprises plural pairs of solid electrodes, each electrode pair having one electrode on each of two sides and having a different ion-selective  
25 layer, at least one porous capillary bridge having the

function as above described, and at least one liquid-distribution porous member provided for each side of plural pairs of solid electrodes so that the member covers at least partially all the electrodes at each  
5 side.

In the accompanying drawings:

Figure 1 is a schematic side view in dis-assembled form showing an embodiment of the device of this invention;

10 Figure 2 is a cross sectional view taken along the plane P of the reassembled embodiment shown in Figure 1;

Figure 3 is an enlarged side sectional view as in Figure 2 showing the device in use;

15 Figure 4 is a side view showing an embodiment of the upper part of the device of Figure 1; and

Figure 5 is a side view showing another embodiment of a liquid distribution member of the device of Figure 1.

In the present invention the member which distributes  
20 the solutions is structurally separated from porous material for forming a bridge. By separating the function of liquid distribution from the function of the bridge, it becomes possible to simultaneously measure the activities of specific ionic components contained in a test solution having various  
25 problems, e.g. difficulty in distribution, such as whole blood, with certainty by an easy operation. Also, by this invention, it has now become possible to measure the electric potential differences of plural pairs of ion-selective electrodes



by only one spotting operation for each of a test solution and a reference solution.

The invention will now be further explained in more detail by referring to the accompanying drawings.

5        Figure 1 is a schematic side view showing an embodiment of the device for determining ionic activity of this invention. In Figure 1 numeral 26 indicates film-shaped ion-selective solid electrodes arranged as paired structures (a single electrode element comprising 10 paired ion-selective electrodes), each pair having an ion-selective layer 26a as an outermost layer (the detailed layer structure of the solid electrodes is not shown in the figure) and each solid electrode pair 26 has terminal portions 27 for electrical connection at 15 both end portions thereof. The plural pairs of solid electrodes 26 (each electrode pair having a different ion-selective layer 26a, 26b or 26c) are placed in a support frame 28 having partitions 29 for electrically insulating each pair of solid electrodes from the other 20 electrode pairs placed therein. A water-impermeable material layer 30 is stuck to the surface of afore-said solid electrode 26 so that the layer 30 uniformly covers the surfaces of all the solid electrodes 26, said layer 30 having liquid supplying apertures 31 capable of 25 supplying the solution to definite portions of each solid electrode in each pair 26 and retaining therein the solution. Two liquid distribution porous members 32,

which are significant features of this invention,  
each connecting the domains corresponding to the  
electrodes 26 on one side having different ion-selective  
layers 26a, 26b and 26c with each other are disposed  
5 between the foregoing plural pairs of solid electrodes.  
A porous bridge, which has a liquid connection at each  
side to all electrodes in one side of pairs of electrodes  
through the liquid distribution porous member upon spot-  
ting of a test solution or a reference solution, is  
10 provided on or between the above described two liquid  
distribution members. The porous bridge will be  
described hereinafter. A liquid storage member or frame  
33 having two liquid storage cells 34 for containing the  
foregoing liquid distribution porous members for storing  
15 a test solution and a reference solution, respectively,  
each cell for each electrode side of the foregoing  
plural pairs of solid electrodes, is disposed on the  
water-impermeable material layer on the plural pairs of  
solid electrodes to make the device rigid and unbendable  
20 together with the foregoing support frame 28. Also, the  
form of the liquid storage cell 34 is not limited to the  
square form as shown in Figure 3 but any form which can  
connect the liquid supplying apertures 31 with each  
other and does not disturb the distribution of the  
25 liquids supplied to the apertures 31 may be employed in  
this invention.

One embodiment of the device of the invention includes a porous capillary bridge 38 formed on and integral with the device, the bridge serving to connect a test solution spotted onto one of the liquid distribution porous members 32 or liquid storage cells 34 with a reference solution spotted onto another liquid distribution porous member 32 or liquid storage cell 34, by the diffusion of both solutions so as to achieve electrical conduction between both electrodes in each pair through both solutions. In another embodiment of the device of the invention, after spotting a test solution and a reference solution onto the foregoing two liquid distribution porous members 32 or liquid storage cells 34, a separate porous bridge 38 is brought into contact with or close to the device so as to allow electrical conduction between both electrodes in each pair through both solutions.

In a preferred embodiment as shown in Figure 1, a cover 35 is formed on the foregoing liquid storage member 33. This is, the cover 35 is formed on the liquid storage member 33, having a set of two apertures for liquid spotting 36 capable of supplying a test solution and a reference solution to the foregoing two liquid distribution porous members 32, respectively, in

the liquid storage cells 34, and several ventilation apertures 37. The cover 35 is shaped so that it covers the whole upper surface of the member 33. In this case, it is preferred that the apertures for liquid spotting 36 and ventilation apertures 37 be positioned as aligned with the positions of the liquid supplying apertures 31 disposed under the cover 35. Onto the upper surface of the cover 35 can be stuck the aforementioned porous capillary bridge 38 composed of a twisted thread or fibers of an organic synthetic polymer at both ends of the bridge; the porosity of said bridge is plugged at both ends thereof by heat sealing or by the use of an adhesive, for connecting the foregoing set of apertures for liquid spotting 36. A practical example of the device of this invention is illustrated in Figure 4 as a sectional view, although the dimensional ratio or relation of the parts shown in the figure is only for reference.

In a practical embodiment of the device of this invention, it is preferred that each film-shaped electrode be a single electrode element comprising paired ion-selective electrodes formed on a common support. Practical examples of such an electrode are described in Japanese Patent Application No. 40398/82, corresponding to GB 8307041, DT P 3309251.6 and FR 83.04220. This is, each pair of solid electrodes is a single electrode element.

comprising paired ion-selective solid electrodes composed of an electrically insulating support having formed thereon, in succession, a conductive metal layer which is skived with a groove at the center by which the metal layer is divided into two electrically isolated portions, a layer of a water-insoluble salt of the metal of the aforesaid conductive metal layer, which is also divided into two portions by the groove together with the conductive metal layer, and a common ion-selective layer. The single electrode element as described above is desirable because the distance between electrodes can be greatly reduced as compared to a pair of electrodes composed of two independently disposed electrodes in a conventional device for determining ionic activity. It is also possible to greatly reduce the distance for distributing a test solution and a reference solution, the length of a bridge between a pair of electrodes, and the amount of both solutions which must be distributed.

When measuring ionic activities using the device for determining ionic activity of the present invention having the foregoing structure, for example, 50 to 60  $\mu$ l of whole blood and the same amount of a reference solution are spotted onto a set of apertures for liquid spotting 36, respectively, formed on a cover 35. As shown in Figure 3 as an enlarged view of an

embodiment of this invention, a spotted solution A is quickly absorbed by a liquid distribution porous member 32 contained in a liquid storage cell 34 of the liquid storage member 33 to be stored in the liquid storage cell  
5 and at the same time is transported by diffusion as shown by arrow a. The liquid distribution porous member 32 retains therein the solution in an amount larger than the liquid-retaining capacity thereof and thus distributes the solution onto the surface of the  
10 electrode 26. It is preferred that the liquid distribution porous member 32 be composed of a loose porous material; if a dense porous material is employed, the liquid spotted thereon forms a liquid film on the liquid supplying apertures 31 and hence the liquid is  
15 reluctant to enter the liquid supplying apertures 31.

If air exists in the inside of the liquid supplying apertures 31, the air prevents the permeation of the liquid into the liquid supplying apertures. In order to remove the air, plural ventilation apertures 37  
20 are formed in the cover 35 covering the liquid storage cells 34 and the air is expelled as shown by arrow B. Each solution thus stored in each liquid storage cell 34 is distributed to each electrode by each liquid distribution porous member 32. On the other hand, the solution  
25 A diffuses also onto the bridge (not shown in Figure 3) connect-

ing the apertures for liquid spotting 36 and is brought into contact with another solution diffused from another side of the bridge to provide an electrical conduction between electrodes in each pair, whereby a potential difference is formed between each pair of electrodes. A potential measuring device (not shown) is connected to the terminal 27 (as shown in Figure 1) which electrically connects each pair of electrodes and thus the ionic activities of different ions are simultaneously or successively determined by one operation. In addition, the amount of the solution supplied to each electrode through each liquid supplying aperture is in the range of 10  $\mu$ l to 20  $\mu$ l.

The following are details of the preferred construction of the parts of the device of the invention.

Solid electrodes used in the present invention can be prepared by conventionally known methods as will be described hereinafter. The support frame 28 may be made of a material as used for an insulating support for a solid ion-selective electrode, which has an electrical insulating property, has a hardness to some extent, and can be easily processed. For example, the support frame may be composed of a sheet of a polymer such as cellulose acetate, polyethylene

terephthalate, polycarbonate of bisphenol A, polystyrene, etc., a glass plate, a ceramic plate, or a paper.

The water-impermeable material layer 30 is, for example, composed of a double sided adhesive film or tape, to  
5 which are stuck the electrodes 26 and the liquid storage member 33. Alternatively, the water-impermeable material layer 30 and the liquid storage member 33 can be stuck using an adhesive or double sided adhesive film. The liquid distribution porous member 32 is composed of a  
10 textile which is woven or knitted from a yarn of hydrophilic natural fibers or organic polymer fibers having at least hydrophilic surfaces, said textile being porous and easily ventilating air to transport a liquid. For example, bandage cloths, gauzes, tricot, clusters of  
15 synthetic polymer fibers, mosquito net cloths of hemp, victoria lawn or cheesecloth, clusters of glass fibers subjected to a hydrophilic treatment or asbestos are preferably used as such materials.

The material used for the liquid distribution  
20 porous members may be treated with a surface active agent. Preferred surface active agents include nonionic surface active agents. Preferred treatment methods with the surface active agents include a method of immersing the liquid distribution member in an aqueous solution of  
25 the surface active agent followed by drying and a method



of spraying an aqueous solution of the surface active agent onto the liquid distribution member followed by drying.

The liquid storage member 33 is composed of a polymer which is the same as the polymer used for the insulating support as described above, such as polystyrene, having relatively large punched apertures or rectangular holes for forming the liquid storage cells 34. The liquid storage member or upper cover can be rendered opaque by the incorporation of a fine titanium oxide powder, etc. The upper cover 35 can be omitted, and in such an embodiment, the liquid storage member is the uppermost part of the device.

The cover 35 has a three-layer structure (as shown in Figure 2) composed of a layer 39 of a polymer which is the same as the foregoing polymer used for the insulating support, such as polyethylene terephthalate film, and double sided adhesive film 48 stuck to both surfaces of the polymer layer 39. The cover 35 also has a set of apertures for liquid spotting 36 and plural ventilation apertures 37. The apertures for liquid spotting 36 and the ventilation apertures 37 have a suitable diameter of, for example, about 3 mm to about 5 mm. However, a larger diameter may be employed for the apertures for liquid-spotting 36 for supplying a

sufficient amount of liquid (10 to 20  $\mu$ l for each electrode surface) to the surfaces of plural pairs of solid electrodes from a set of apertures for liquid spotting. The ventilation apertures 37 may be smaller than the aforesaid diameter and the number of the ventilation apertures 37 may be increased. Furthermore, although a set of apertures for liquid spotting 36 are formed at the center portion of the cover 35 in the embodiment shown in Figure 1, they may be positioned at another area, i.e., in the area of the ventilation apertures 37.

In this embodiment, a construction is employed wherein a twisted thread capillary bridge composed of fibers (hereinafter, the bridge is simply referred to as a thread bridge) is formed on the upper cover 35. However, the thread bridge may be formed on or under the interlayer of the upper cover having a three-layer structure.)

The twisted thread can be composed of natural fibers or organic synthetic polymer fibers or filaments such as cotton fibers, silk fibers, polyamide (nylon) fibers, acetate rayon fibers, cured or partially acetalized polyvinyl alcohol fibers. It is preferably comprised of polyamide (nylon) fibers or silk.

In a preferred embodiment of the foregoing cover 35 and liquid storage member 33, the cover and the liquid storage member are molded in a unitary upper member 40 as shown in Figure 4. This member 40 shown in  
5 Figure 6 has a set of apertures for liquid spotting 36 and plural ventilation apertures 37 through both surfaces thereof, a V-shaped groove 41 formed on upper surface for connecting the two apertures for liquid spotting 36, and a twisted thread bridge 38 is embedded  
10 in the V-shaped groove so that the bridge is disposed on the groove, in the inside of the groove, or on the bottom of the groove. The molded member 40 has two liquid storage cells 34 in the inside thereof as described above and the foregoing apertures for liquid  
15 spotting 36 and ventilation apertures 37 are opened in the liquid storage cells 34. In the liquid storage cells 34 are placed the liquid distribution porous members 32 as described above. The unitary structure of the liquid storage member, the cover, and the thread bridge as  
20 described above can simplify the production steps of the device of this invention.

In the foregoing embodiments, a twisted thread bridge composed of fibers is employed as the porous capillary bridge but the porous capillary bridge used in  
25 this invention is not limited to such a twisted thread

bridge and all the conventionally known porous capillary bridges may be used in this invention. For example, a porous capillary bridge composed of a mixed fiber paper, previously proposed by the inventors (Japanese Patent Application (OPI) No. 14050/83, corresponding to GB 2,106,253A) can be used in this invention. The use of the twisted thread bridge as described hereinbefore is desirable in that the diffusion of a test solution and a reference solution permeating into the bridge can be limited to one direction and the amount of solutions to be spotted may be reduced. However, the particularly important point of using such a twisted thread bridge is that the bridge can be used for a test solution having a high viscosity, such as whole blood. Furthermore, the employment of a liquid distribution porous member, liquid storage cells, and/or apertures for liquid spotting makes it possible to use a separate bridge or a bridge which is not stuck or fixed onto the surface of the device. That is, if any liquid storage means as described above is or are employed, the electrical conduction of a test solution and a reference solution can be attained by placing a separate bridge on both solutions after spotting the liquids into the apertures for liquid spotting for contacting both liquids therewith. For the porous capillary bridge used in this case, there

is a fibrous twisted thread or a filter paper held at the lower edges of a support having a  $\sqcap$ -shaped sectional form across the space of the support formed between two side walls. Another embodiment of the porous capillary  
5 bridge used in this invention is the bridge used for an ionic activity determining device disclosed in Japanese Patent Application No. 69933/82, wherein a lid-form member having a porous capillary bridge is equipped thereto in such a manner that the lid is in an opened  
10 state but is closed after spotting liquids for contacting both liquids.

In another embodiment of this invention, a liquid distribution porous member which also acts as a liquid storage member may be employed. It is possible  
15 to use a member 47 shown in Figure 5, which is composed of a sheet of a fibrous material such as that used for the foregoing liquid distribution porous member. This sheet is plugged at the frame portion 46 leaving two liquid storage cell portions 49 unplugged for preventing  
20 the liquids stored in the liquid storage cell portions 49 from diffusing into the frame portion 46. Plugging of the foregoing fibrous sheet can be performed by heat welding, by the use of a hot melt type adhesive or by printing using a hardenable hydrophobic ink.

The solid electrodes used in this invention can be prepared by a known method such as disclosed in the aforesaid Japanese Patent Appln. No.40398/82. This is, a thin conductive metal layer is formed on a proper electrically insulating support, such as a polymer sheet or film. The thin metal layer can be formed using a vacuum vapor deposition method, electroless plating or chemical plating, etc. A support having a thickness of about 50  $\mu\text{m}$  to about 500  $\mu\text{m}$ , preferably about 80  $\mu\text{m}$  to about 300  $\mu\text{m}$  and having a smooth surface is desirably used. The thickness of the metal layer is about 50 nm to about 50  $\mu\text{m}$ , preferably about 400 nm to about 10  $\mu\text{m}$ . As the metal for the conductive metal layer, a metal having electrical conductivity and stability in the air, such as silver, platinum, palladium, gold, nickel, copper, aluminium, indium, etc., can be used. In order to provide terminals for electrical connection at both end portions of each thin metal layer, masking is applied to the metal layer formed on the support.

Useful masking methods include a method of masking the end portions by coating a known resist, a method of coating the end portions with a liquid resist which can be removed by an alkali as disclosed in Research Disclosure, No. 19445 (June 1980), a method of

masking by forming a thin vapor-deposition layer of nickel or chromium of 5 nm to 20 nm in thickness as disclosed in U.S. Patent 4,259,164, a method of masking by forming a thin vapor-deposition layer of palladium of 5 1.5 nm to 15 nm in thickness as disclosed in Japanese Patent Application (OPI) No. 186163/82 or of indium of 3 nm to 20 nm as disclosed in Japanese Patent Application (OPI) No. 33159/83, and a method of masking using a liquid resist having a film-forming property and a 10 peeling-off property after drying, such as a liquid resist composed of polyvinyl chloride as the main component.

After masking the thin metal layer at the terminal portions for electrical connection, the metal 15 layer at the unmasked portion is, if necessary, converted into a water-insoluble salt of the metal or alternatively a water-insoluble salt of the metal may be formed on the thin metal layer at the unmasked portion. When the metal of the thin metal layer is silver, the typical 20 water-insoluble salt of the metal is a halide of the metal, i.e., a silver halide. The metal salt layer is formed by coating or treating the metal layer with a composition containing an oxidizing agent (and a halide ion when the oxidizing agent does not contain a halide 25 ion). The oxidizing agent can be applied to a silver

layer by a conventional method such as a roll coating method, a dip coating method, a lamination method, and a brush coating method. The oxidizing agent may be used as a solution thereof in an aqueous solution containing  
5 an acid such as hydrochloric acid.

Examples of useful oxidizing agents include  $\text{KCrO}_3\text{Cl}$ ,  $\text{K}_3[\text{Fe}(3+)(\text{CN})_6]$ ,  $\text{KMnO}_4$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{NH}_4\text{VO}_3$ ,  $(\text{NH}_4)_2[\text{Ce}(4+)(\text{NO}_3)_6]$  and  $\text{Fe}(3+)_2(\text{OOC-COO})_3$ . Preferred oxidizing agents are  $\text{KCrO}_3\text{Cl}$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ , and  
10  $\text{K}_3[\text{Fe}(3+)(\text{CN})_6]$ . These oxidizing agents may be used alone or in combination. Useful oxidizing agents are described in more detail in, for example, Handbook of Chemistry and Physics, 50th Edition, pages D109-114, published by The Chemical Rubber Company, 1969.

15 The amount of the oxidizing agent used depends upon the thickness of the silver halide layer to be formed but is preferably used in an amount within the range of 0.01 to 2.0 g/m<sup>2</sup>. The silver halide to be formed as a silver halide layer may be silver chloride,  
20 silver bromide, or silver iodide. Such a silver halide layer may be also formed by an electrolytic method or other method as disclosed in U.S. Patent 4,214,968. A metal/metal salt (in particular, Ag/AgX, wherein X is a halogen atom) reference electrode can be prepared by  
25 using the technique usually used for producing photo-



graphic films as disclosed in U.S. Patent 4,214,968, Column 11.

On the metal salt layer thus formed by the method described above can be formed, if necessary, a reference electrolyte layer for the stabilization of electric potential by a known method. The formation of the reference electrolyte layer can be performed using the technique described in, for example, Research Disclosure, No. 16113 (September 1977), U.S. Patent 4,214,968, and Japanese Patent Application (OPI) No. 17852/82.

An ion-selective layer is a layer capable of selectively responding to a specific ion and the ion-selective layer can be formed by a known method. For example, an ion-selective layer may be formed by coating a solution prepared by dissolving an ion carrier in an ion-carrier solvent together with a polymer binder in a solvent on the water-insoluble salt layer or the reference electrolyte layer followed by drying by removing a solvent for polymer binder. The concentration of the ion carrier is generally 0.05 to 10 g/m<sup>2</sup> and the thickness of the ion-selective layer is about 3 μm to about 125 μm, preferably 5 μm to 50 μm.

The ion-selective layer used in this invention must be water-insoluble since both the test solution and reference solution employed in this invention are aqueous

solutions. The ion-selective layer may be either hydrophilic or hydrophobic if the layer is water-insoluble but it is preferred that the layer be hydrophobic.

The material constituting the solid electrodes  
5 in this invention may be any material generally used for electrodes in this field.

A most typical ion-selective layer is composed of an ion carrier, an ion carrier solvent, and a hydrophobic organic polymer binder (or a matrix composed of  
10 a hydrophobic organic polymer binder). Examples of ion carriers include valinomycin, cyclic polyethers, tetralactones, macrolide actins, enniatins, monensin, esters of monensin, gramicidine, nonactin, tetraphenyl borate and cyclic polypeptide.

15 Examples of ion carrier solvents include trimellitates, bromophenyl phenyl ether, 3-methoxyphenyl phenyl ether, 4-methoxyphenyl phenyl ether, dimethyl phthalate, dibutyl phthalate, dioctyl phthalate, dioctylphenyl phosphate, bis(2-ethylhexyl) phthalate, octyl-  
20 diphenyl phosphate, tritolyl phosphate and dibutyl sebacate.

Examples of hydrophobic organic polymer binders include hydrophobic natural or synthetic polymers capable of forming films, such as cellulose esters, polyvinyl  
25 chloride, polyvinylidene chloride, polyacrylonitrile,

polyurethane, polycarbonate and vinyl chloride-vinylidene chloride copolymers.

For the ion carriers, ion carrier solvents, hydrophobic organic polymer binders, solvents for polymer binder and ion-selective layers composed of these materials, the materials and techniques described in, for example, U.S. Patents 4,053,381, 4,171,246, 4,214,968 and 4,115,209, and Research Disclosure, #16113 (September, 1977) can be used in this invention.

As the material for the ion-selective layer, an ion exchanger may be also used. The ion exchanger used in this invention may be cationic or anionic. Proper ion exchangers and ion-selective layers using these ion exchangers are described in detail in, for example, U.S. Patent 4,115,209.

When the ion to be measured is  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $HCO_3^-$ , etc., the use of an ion-selective layer is inevitable but when the ion to be measured is  $Cl^-$ , the metal layer of the solid electrodes is composed of silver, and the water-insoluble metal salt layer is composed of silver chloride, the use of an ion-selective layer being unnecessary. In the latter case, a layer composed of the material described in U.S. Patents 4,199,411 and 4,199,412, such as a cellulose ester (e.g., cellulose acetate, cellulose acetate butyrate,

cellulose acetate propionate, hydrolyzed cellulose acetate butyrate, and mixed esters thereof) or the latex described in, for example, U.S. Patents 4,199,362 and 4,256,827 may be formed as an ion-permeable protective layer having permeability with respect to the ion to be measured in place of forming an ion-selective layer. In this invention, such ion-permeable protective layers are included in the term "ion-selective layers".

Each single electrode thus prepared as described above is used as a pair of two electrodes. It is important for a pair of electrodes to have the same electrochemical characteristics. In a preferred embodiment of this invention, a pair of electrodes each having the same properties can be easily constructed by the method proposed in our aforesaid Japanese Patent Application No. 40398/82 without requiring the application of a specific insulating means. That is, according to the foregoing method, a single electrode element comprising paired electrodes is prepared by forming functional layers on an insulating support and skiving a groove between each metal layer of the functional layers before, preferably, forming the uppermost ion-selective layer on the functional layers. Skiving can be easily performed using a sharp edged tool such as a knife, etc., the electrical insulation between the

separated metal layers (between electrodes) can be completely attained, and further in the electrodes the occurrence of shorting thereof due to the flowing liquid oozed from a bridge, etc., can be prevented by covering  
5 the inner edges of the functional layers of the electrodes with a continuous uppermost ion-selective layer.

The electrodes or single electrode elements thus prepared are cut into one electrode or one element.  
10 Then, plural pairs of electrodes or plural elements, each pair or element having a different ion-selective layer, are mounted in a support frame, and a water-impermeable adhesive layer, such as double sided adhesive film having punched liquid supplying apertures and  
15 ventilation apertures, is stuck to the pairs of electrodes or single electrode elements. A liquid storage member which contains liquid distribution porous members and is then formed in a unitary body with an upper cover is stuck to the surface of the  
20 adhesive film to provide the ionic activity determining device of this invention.

Using a device of this invention, ionic activities of different kinds of ions can be simultaneously determined by spotting a test solution and a  
25 reference solution each in one operation and measuring

an electrical potential difference of each pair. Further, since a thread bridge is used, a test solution having a high viscosity, such as whole blood can be tested with respect to its ion activity.

5           The invention will now be further described by the following examples.

#### ELECTRODE PREPARATION 1

On a polyethylene terephthalate film (PET film) of 180  $\mu\text{m}$  in thickness was vapor-deposited a silver  
10 layer having a thickness of 500 nm and the film was cut into pieces of 28 mm in width and 1.8 m in length. A groove of 70  $\mu\text{m}$  in depth was formed by skiving the silver metal layer on the PET film at the longitudinal center line using a knife. Both edge portions (3 mm  
15 each in width) of the silver layer formed on the film were coated with a solution of a vinyl chloride-vinyl acetate copolymer in a mixed solvent of toluene and methyl ethyl ketone (a film-forming liquid resist for masking capable of being removed by peeling-off after  
20 drying) followed by drying to form protective layers each having a thickness of 30  $\mu\text{m}$ . The film was immersed in an oxidative chlorination treatment solution containing 60 mmole/liter of hydrochloric acid and 12 mmole/liter of dichromic acid for 90 sec. at 30°C, washed with  
25 water, and dried to provide solid film-shaped Ag/AgCl reference electrodes.

Then, a solution of 0.9 g of a vinyl chloride-vinyl acetate copolymer (VYNS), 1.35 g of methyltrioctyl ammonium chloride, and 0.05 g of didodecyl phthalate dissolved in 5 g of methyl ethyl ketone was coated on the AgCl layer of the foregoing solid film-shaped reference electrodes thus formed in the aforesaid immersion procedure and dried to form a chloride ion-selective layer having a thickness of 25  $\mu\text{m}$ .

Thereafter, the mask layers coated on the edge portions were peeled off to expose the silver metal layer at the terminal portions for electrical connection. The ion-selective solid electrode film was cut into pieces each having a width of 6 mm to provide film-shaped ion-selective electrodes for determining ionic activity of chlorine ion.

#### ELECTRODE PREPARATION 2

By following the same procedure as in Preparation 1 using 0.9 g of VYNS, 33 mg of valinomycin, 1.7 g of dioctyl phthalate, and 5 g of methyl ethyl ketone, an ion-selective layer for potassium ion was formed in place of the chloride ion-selective layer in Example 1. The thickness of the potassium ion-selective layer was 30  $\mu\text{m}$ . Then, in the same way as in Example 1, solid film-shaped ion-selective electrodes for determining ionic activity of potassium ion were prepared.

### ELECTRODE PREPARATION 3

By following the same procedure as in Preparation 1 using 0.9 g of VYNS, 0.4 g of methylmonensin, 1.8 g of dioctyl sebacate, and 5 g of methyl ethyl ketone as a coating composition, an ion-selective layer for sodium ion was formed in place of the chlorine ion-selective layer in Example 1. The thickness of the sodium ion-selective layer was 25  $\mu$ m. In the same manner as in Example 1, solid film-shaped ion-selective electrodes for determining ion activity of sodium ion were prepared.

### EXAMPLE 1

By following similar procedures as in Preparations 1 to 3, single electrode elements comprising paired ion-selective electrodes, each having a skived silver layer, for ions,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  were prepared. On a heat-molded high impact polystyrene support frame 28 of 28 mm in length and 24 mm in width as shown in Figure 3, were disposed the foregoing three elements (three pairs) each having a width of 6 mm and a length of 28 mm.

A plastic sheet 35 having 2 sets of ventilation apertures 37 and one set of apertures for liquid spotting 36 and a plastic frame or sheet 35 having two liquid storage cells 34 as shown in Figure 3 were prepared, and both sheets were stuck to each other using a double sided adhesive film to provide an upper cover



or upper frame. Then, two medical gauze pieces 32 each having a width of 4.5 mm and a length of 18 mm were inserted in the liquid storage cells 34, respectively, of the cover so that the upper surfaces of the gauze pieces 32 uniformly covered the apertures 36 and 37 of the plastic sheet 33 at the lower side thereof. In this device, one aperture of a set of apertures for liquid spotting 36 was for a reference solution and another aperture thereof was for a test liquid.

10 Then, a water-impermeable material member 30 composed of a double sided adhesive film having six punched liquid supplying apertures 31 were stuck to the whole lower surface (excluding four corner portions or four marginal portions) of the foregoing upper cover so that the positions of the liquid supplying apertures 31 15 matched the positions of the apertures for liquid spotting 36 and ventilation apertures 37. Then, a bridge 38 composed of a twisted thread of nylon fibers was disposed on the upper or front surface of the cover and over a set of apertures for liquid spotting 36 and 20 both ends of the bridge were fixed by welding to the surface of the cover by means of a heated soldering iron.

Then, a parting paper at the lower or back side of the double sided adhesive film 30 stuck to the 25 cover was peeled off and the cover with the adhesive

tape 30 was stuck to the three single electrode elements 26 placed in the support frame 28. Finally, the cover was fixed to the support frame at the four corner portions or four marginal portions having no adhesive film by heat welding using ultrasonic wave to provide a one-tip type device for determining ionic activities of ions,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ .

#### EXAMPLE 2

Using the ionic activity determining device prepared by the method described in Example 1, the potentials based on the ions,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in control serum were measured. Versatol<sup>®</sup> was used as a reference solution and Versatol A<sup>®</sup> and Versatol AA<sup>®</sup> were used as test solutions. The reference solution and the test solution were spotted each once to the central apertures for liquid spotting of the ion activity determining device prepared by the method shown in Example 1 at an amount of 60  $\mu\text{l}$  each. The potential differences after 2 minutes at 25°C were measured by means of Model 901-type Ion Analyzer, made by Orion K.K. The results obtained are shown in Table 1.

#### EXAMPLE 3

The same procedure as in Example 2 was followed while spotting once as a test solution 60  $\mu\text{l}$  of whole blood from a rabbit obtained using heparin lithium as an

anti-coagulant onto the central aperture for liquid spotting and the potential differences after 2 minutes were measured by the same manner as in Example 5. The results are shown in Table 2-A.

5 Furthermore, the whole blood of the rabbit was centrifuged to remove erythrocyte and by using the blood plasma as a test solution, the measurement by one spotting was performed as in Example 5. The results obtained are shown in Table 2-B.

10

TABLE 1

<u>Measured Ion</u>	<u>Versatol A</u>		<u>Versatol AA</u>	
	<u>Measured Value (mV)</u>	<u>Indicated Value (mEq/l)</u>	<u>Measured Value (mV)</u>	<u>Indicated Value (mEq/l)</u>
Na <sup>+</sup>	-3.0	126	+1.3	151
K <sup>+</sup>	+10.8	7.3	-10.2	3.1
Cl <sup>-</sup>	+2.8	91	-1.5	108

15

TABLE 2

<u>Measured Ion</u>	<u>Measured Value (mEq/l)</u>	
	<u>A (whole blood)</u>	<u>B (plasma)</u>
Na <sup>+</sup>	145	147
K <sup>+</sup>	5.3	5.6
Cl <sup>-</sup>	109	107

EXAMPLE 4

By following the same procedure as in Example 1 using a medical bandage treated with a nonionic surface active agent (an aqueous solution of polyoxyethylene nonylphenyl ether) as the liquid distribution porous member in place of the medical gauze in Example 1, an ionic activity determining device was prepared.

When the same measurement as in Example 2 was performed using the device with respect to ions,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  in control serum, almost the same results as in Example 5 were obtained.

EXAMPLE 5

By following the same procedure as in Example 1 using Rapia S<sup>®</sup> (tricot, made by Teijin Limited) in place of the medical gauze, an ionic activity determining device was prepared.

When the same measurement as in Example 2 was performed using the foregoing device with respect to ions,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in control serum, almost the same results as in Example 2 were obtained.

Claims:

1. A device for determination of ionic activity in a liquid analyte, which comprises:
  - at least two pairs of solid electrodes (26) each
  - 5 having as its outer layer an ion-selective layer (26a, b, c) responsive to a specified ion,
  - at least two porous members (32) each in contact with at least part of the surface of the ion-selective layer of each pair of electrodes, the porous member being
  - 10 capable of supplying said liquid analyte and a reference solution to the electrodes, and
  - a porous bridge (38) disposed over said porous members (32) to allow, during use of the device, electrical conduction between both electrodes in each pair through
  - 15 both solutions.
2. A device as claimed in Claim 1, wherein said bridge (38) is composed of a twisted thread of fibres.
3. A device as claimed in Claim 1 or 2, which also includes a cell (34) for storage of the liquid and which
- 20 has cavities for said porous members (32).
4. A device as claimed in Claim 3, wherein a separating element (30) between the cell (34) and electrodes (26) has apertures (31) for supply of liquid to defined parts of each electrode (26) in each pair.
- 25 5. A device as claimed in any preceding claim, which also includes a cover (35) having two apertures (36) for supply to two porous members (32) of the analyte and

reference solution respectively, and the porous bridge (38) bridges over said apertures (36).

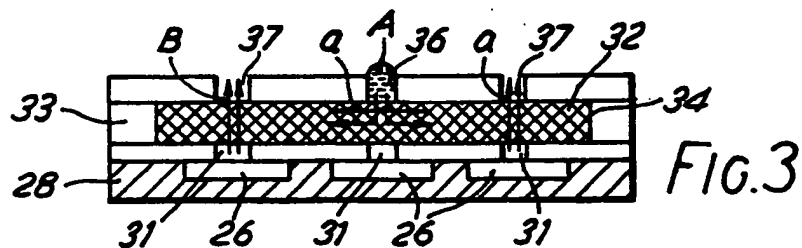
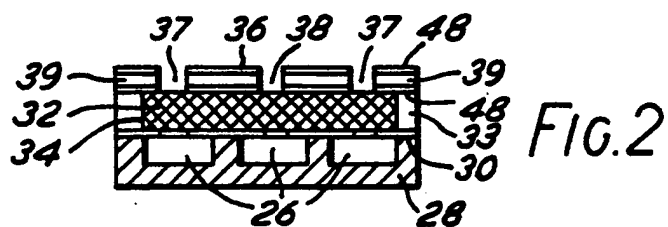
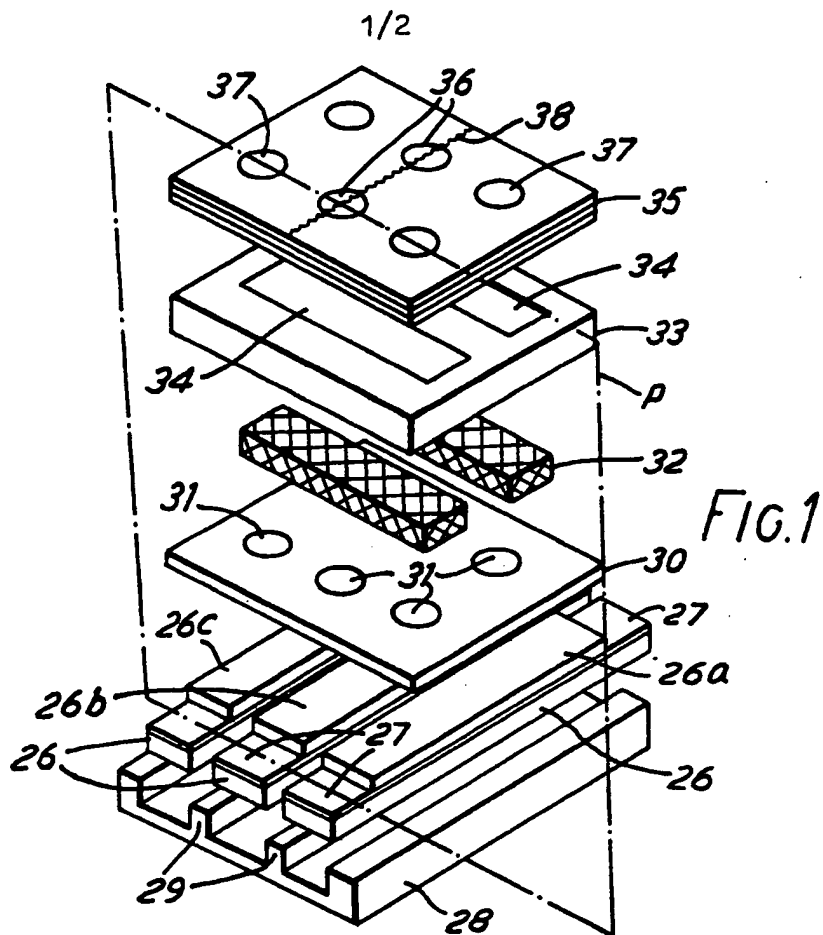
7. A device as claimed in any of Claims 1 to 4, which also includes a multilayer cover (35) having two  
5 apertures (36) for supply to two porous members (32) of the analyte and reference solution respectively, and the porous bridge (38) is retained between the layers of the cover.

8. A device as claimed in any preceding claim, wherein  
10 the liquid distribution member (47) also acts as a liquid storage member.

9. A device as claimed in any preceding claim, wherein each pair of solid electrodes (26) is a single electrode element comprising paired solid ion-selective electrodes  
15 comprising a common electrically insulating support having formed thereon a conductive metal layer which is divided into two electrically isolated portions by a groove, a layer of a water-insoluble salt of said metal, and a common ion-selective layer (26a, b, c).

20 10. A method of determining ionic activity in a liquid analyte, by means of a device as claimed in any preceding claim, which comprises applying the liquid analyte and a reference solution to the respective porous members (32), and applying a potential measuring device to a terminal (27)  
25 of each electrode, with the porous bridge (38) allowing electrical conduction between both electrodes in each pair.

11. A d v i e as claimed in any of Claims 1 to 9 for  
determining ionic activity, composed of plural pairs of  
solid electrodes (26) each pair having an ion-selective  
layer selectively responsive to a specific predetermined  
5 ion as the outermost layer of the solid electrodes and one  
porous bridge (38) formed thereon for achieving, after  
supplying a test solution and a reference solution to said  
pairs of solid electrodes (26), electrical conduction between  
both electrodes in each said pair by the permeation of  
10 the solutions through the bridge (38), and each electrode  
pair has one electrode on each of two sides and has a  
different ion-selective layer, at least one liquid  
distribution porous member (32) being provided for each  
said side of plural pairs of solid electrodes so that  
15 said porous member (32) covers at least partially all  
the electrodes at each said side, and said bridge (38) is  
disposed on or between said liquid distribution members.





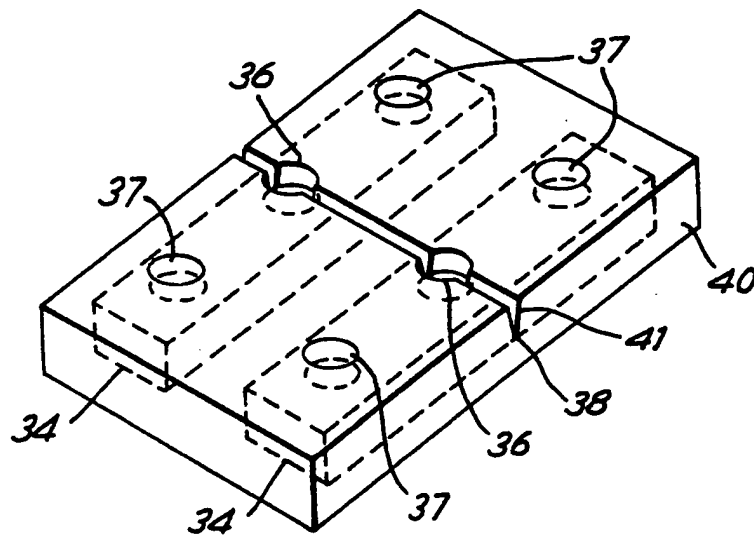


FIG. 4

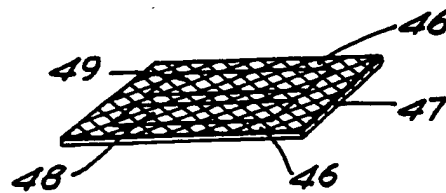


FIG. 5



European Patent  
Office

# EUR PEAN SEARCH REPORT

0095946

Application number

EP 83 30 3193

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 7)
A	US-A-4 250 010 (FUJI PHOTO)  * Figures 1-3; column 4, lines 59-68; column 5, column 6, lines 1-46 *	1,8,10,11	G 01 N 27/30 G 01 N 27/28
A	--- EP-A-0 007 794 (EASTMAN KODAK)  * Figures 1, 2; pages 3-7 *	1-5,7,10,11	
A	--- EP-A-0 010 457 (EASTMAN KODAK)  * Figure 12; page 15, lines 21-36; pages 16-17; page 18, lines 1-10 *	1-3,5,7,10,11	
A	--- EP-A-0 021 798 (EASTMAN KODAK)  * Figures 1-3; pages 5-8 *	1-3,5,10,11	TECHNICAL FIELDS SEARCHED (Int. Cl. 7)
A	--- Patent Abstracts of Japan vol. 6, no. 82, 20 May 1982 & JP-A-57-017852 (Cat. D)  -----	9	C 25 B 9/00 C 25 B 9/02 G 01 N 27/07 G 01 N 27/26 G 01 N 27/28 G 01 N 27/30 G 01 N 27/46 G 01 N 27/56 G 01 N 27/58 G 01 N 31/04 G 01 N 33/48
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 16-08-1983	Examiner DEVEZA R.R.
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			